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Obesity-induced changes in lipid mediators persist after weight loss

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Abstract

Background—Obesity induces significant changes in lipid mediators, however, the extent to which these changes persist after weight loss has not been investigated.

Subjects/Methods—We fed C57BL6 mice a high fat diet to generate obesity and then switched the diet to a lower fat diet to induce weight loss. We performed a comprehensive metabolic profiling of lipid mediators including oxylipins, endocannabinoids, sphingosines and ceramides in key metabolic tissues including adipose, liver, muscle, hypothalamus and plasma.

Results—We found that changes induced by obesity were largely reversible in most metabolic tissues but the adipose tissue retained a persistent obese metabolic signature. Prostaglandin signaling was perturbed in the obese state and lasting increases in PGD₂, downstream metabolites 15-deoxy PGJ₂ and delta-12-PGJ₂ were observed after weight loss. Furthermore, the enzyme responsible for PGD₂ synthesis (hematopoietic prostaglandin D synthase, HPGDS) was increased in obese adipose tissues and remained high after weight loss. We found that inhibition of HPGDS

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over the course of 5 days resulted in decreased food intake in mice. Increased HPGDS expression was also observed in human adipose tissues compared with lean individuals. We then measured circulating levels of PGD₂ in obese patients before and after weight loss and found that while elevated relative to lean subjects, levels of this metabolite did not decrease after significant weight loss.

Conclusions—These results suggest that lasting changes in lipid mediators induced by obesity, still present after weight loss, may play a role in the biological drive to regain weight.

Introduction

In America, an estimated 45 million people attempt to lose weight each year (1) and although many achieve short term success, sustained maintenance of reduced body weight is rarely achieved (2–9). A successful weight loss program incorporating diet and lifestyle changes may lead to 10% reduction in body weight (10), but remarkably 75–95% of people regain this weight (2, 9, 11–14). The driving forces behind weight regain include both biological and psychological factors (4, 15, 16). Weight loss is associated with compensatory decreases in energy expenditure, which opposes the maintenance of the lower weight and promotes weight regain (17–21). Furthermore, changes in circulating levels of peripheral modulators important in appetite regulation, including leptin, peptide PYY, cholecystokinin, insulin, ghrelin gastric inhibitory polypeptide, as well as hunger, remain significantly different from baseline one year after weight loss and play an important role in weight regain (22).

Obesity induces significant changes in the metabolome (23–27), however, the extent to which these changes persist after weight loss has not been investigated. We fed C57BL/6 mice a high fat diet (60% calories from fat, HFD) to generate obesity and then switched the diet to a lower fat diet (10% calories from fat, LFD) to induce weight loss (28). In this study we have used targeted metabolomics to study changes in lipid mediators in insulin target tissues (liver, adipose, muscle, hypothalamus) and plasma, in obesity and after weight loss in mice. We performed comprehensive metabolic profiling of lipid mediators including oxylipins, endocannabinoids, sphingosines and ceramides that have been implicated in obesity associated co-morbidities including insulin resistance, diabetes and cardiovascular disease (26, 29–42).

Oxylipins are potent bioactive metabolites that are involved in inflammatory signaling and play important roles in obesity-induced inflammation and insulin resistance (26, 34, 35, 39–41, 43). Oxylipins (eicosanoids, docosanoids, and octadecanoids) are derived from the oxygenation of polyunsaturated fatty acids (PUFAs) by three families of enzymes COX, lipoxygenase (LOX) and cytochrome p450 (CYP) (43). The 12-LOX derived oxylipins are induced in the obese state and studies have shown these oxylipins play an important role in mediating inflammation and result in adipocyte dysfunction (44). The endocannabinoid system plays an important role in energy storage, nutrient transport and insulin sensitivity (29). In the CNS endocannabinoids stimulate food intake through their interactions with the leptin-regulated neurocircuitry and can work as neuromodulators able to directly regulate and release classical neurotransmitters (45, 46). Endocannabinoids act on peripheral tissues

including adipose, liver and muscle and overactive endocannabinoid tone is associated with obesity (29, 30, 36–38). Ceramides are bioactive sphingolipids that accumulate in obesity resulting in the development of insulin resistance (31–33, 42, 47). Ceramides are synthesized by a family of six ceramide synthases (CerS) in mammals, which produce ceramides with different *N*-linked acyl chains of various lengths. Inhibition of ceramide synthesis has been shown to improve systemic metabolism and obesity-induced insulin resistance (33, 42).

While many studies have identified changes in lipid mediators in obesity, the extent to which weight loss reverses these obesity-induced changes has not yet been studied. The mechanisms driving weight rebound are complex and largely unknown, therefore, understanding the barriers to maintaining weight loss are crucial for the prevention of relapse to obesity. We hypothesized that some metabolites induced or repressed in the obese state remain so even after weight loss and these “persistent” metabolites may play a role in driving weight regain. Metabolites that are induced by obesity and not changed upon weight loss will represent therapeutic targets for the maintenance of weight loss.

Materials/Subjects and Methods

In vivo mouse studies

All experiments were approved by and conducted in accordance with the Institutional Animal Care and Use Committee (IACUC) at the University of California, San Diego. Male C57BL6 mice were fed normal chow until 12 weeks of age and then randomly divided into three groups termed LFD (low fat diet, 10% fat; D12450B, Research diets), HFD (high fat diet, 60% fat; D12492, Research Diets) and SWD (switch diet from HFD to LFD). The LFD and HFD group were fed *ad libitum* for 18 weeks. The SW group were fed HFD for 9 weeks and then switched to LFD for a further 9 weeks after which their body weights had returned to a similar mass as the LF group (n=8 per group). Group sizes were selected based on previous similar studies (28). All groups were sacrificed at 30 wks of age and the tissues (hypothalamus, liver and epididymal adipose tissue) dissected and frozen immediately in liquid nitrogen. Glucose Tolerance tests (GTTs) were performed at 3 and 8 weeks after the diet switch as previously described (48). Briefly, mice were fasted for 6 hrs and then injected with glucose (ip, 1 g/kg dose) and blood samples drawn at 10, 30, 60 and 90 mins to monitor glucose excursion over time. A selective inhibitor of hematopoietic prostaglandin D synthase (HQL-79, 30 mg/kg, (Cayman Chemical, Ann Arbor, MI) or vehicle control (saline) was administered by oral gavage for 5 consecutive days to C57BL6 mice fed HFD 8 wks, from 12 wks of age. Body weight and food intake was measured daily at 10 AM prior to gavage and at the end of the study mice were sacrificed and plasma and adipose tissue collected, n=12 mice per group.

Sample extraction

Oxylipins, endocannabinoids, and ceramides were isolated using a 96-well Ostro™ Pass Through Sample Preparation Plate (Waters Corp, Milford, MA) n=6 samples per tissue/per group. For plasma, 50 µL of sample was added to the plate wells and spiked with a 5 µL anti-oxidant solution (0.2 mg/ml solution BHT/EDTA in 1:1 methanolwater) and 10 µL 1000

nM deuterated surrogates. Next, isopropanol w/ 10 mM ammonium formate and 1% formic acid (150 μ L) was added and aspirated three times to mix. Following this step, the mixture was eluted into glass inserts, dried, and reconstituted in the same manner as the tissue samples (as described below). Tissue samples were pulverized (Liver ~20–25 mg, adipose 6–10 mg, muscle 6–10 mg hypothalamus ~0.5–2.5 mg) and added to 2 mL polypropylene tubes spiked with a 5 μ L anti-oxidant solution (0.2 mg/ml solution BHT/EDTA in 1:1 methanol:water) and 10 μ L of 1000 nM deuterated surrogates in methanol. A total of 50 μ L of methanol was added and the tube was placed in a Geno/Grinder 2000 (SPEX Sample Prep, Metuchen, NJ) for 30 sec at 1350 rpm. An additional 550 μ L isopropanol w/ 10 mM ammonium formate and 1% formic acid and 100 μ L water was added and the tube was placed in a Geno/Grinder for an additional 30 sec before being centrifuged at 10,000g for 5 min at room temp. Supernatants were transferred into Ostro Plate wells and eluted into glass inserts containing 10 μ L 20% glycerol in methanol by applying a vacuum at 15 Hg for 10 min. Eluents were dried by vacuum centrifugation in a Genvac EZ-2 (SP Scientific, Stone Ridge, NY) for 35 min at the medium BP setting, before switching to an aqueous setting for an additional 35 min. Once dry, samples were re-constituted with the internal standard 1-cyclohexylureido, 3-dodecanoic acid (Cayman Chemical) and 1-phenylureido, 3-Hexanoic acid (gift from B.D. Hammock, University of California, Davis) at 100 nM (50:50 methanol:acetonitrile), vortexed 1 min, transferred to a spin filter (0.1 μ m PVDF membrane, Millipore, Billerica, MA), centrifuged for 3 min at 6°C at <4500 g (rcf), before transfer to 2 mL LC-MS amber vials. Extracts were stored at –20°C until analysis by ultraperformance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). The internal standards were used to quantify the recovery of surrogate standards.

Lipid mediator analysis

Analytes were separated using a Waters Acquity UPLC (Waters, Milford, MA) with a solvent gradient modified from previously published protocols for oxylipins (40), endocannabinoids (39), and ceramides (49). Samples were held at 10°C. Separated residues were detected in independent injections by negative mode electrospray ionization for oxylipins and positive mode electrospray ionization for endocannabinoids and ceramides using multiple reaction monitoring on an API 4000 QTrap (AB Sciex, Framingham, MA, USA). Analytes were quantified using internal standard methods and 5 to 7 point calibration curves ($r^2 = 0.997$). Calibrants and internal standards were either synthesized [10,11-DHN, 10,11-DHHep, 10(11)-EpHep] or purchased from Cayman Chemical, Avanti Polar Lipids Inc. (Alabaster, AL) or Larodan Fine Lipids (Malmo, Sweden), unless otherwise indicated. Data was processed using AB Sciex MultiQuant version 3.0.2. Lipid mediator data from this study can be accessed from <http://www.metabolomicsworkbench.org>, data set refs ST000593 and ST000594.

Human samples

Plasma PGD2 levels were determined by ELISA (Cayman chemicals, MI) in samples obtained from obese patients before and after bariatric surgery. Specifically, blood samples were collected at the following time-points: 1) Plasma samples were obtained 3 weeks prior to surgery, after which patients committed to a stringent three week 800 cal/day diet regiment. 2) Plasma samples were collected on the day of surgery, at which time the patients

had lost on average 8.7 kg due to the diet regime. 3) Plasma samples were also collected 6 and 12 months after surgery, at which time the patients had lost on average 37.3 kg and 46.7 kg, respectively. These human samples were obtained from a larger study ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02322073) NCT02322073) in agreement with the principal investigator, V. Wallenius. The Regional Ethical Review Board (Gothenburg, Sweden), approved all study procedures (Dnr 682-14) and all patients were enrolled in accordance with the Helsinki Declaration. Written informed consent was obtained from all participants included in this study.

Adipocyte and stromal vascular cell isolation from adipose tissue

Adipocytes and stromal vascular cells were isolated as previously described (50). Briefly, visceral adipose tissue was mechanically chopped and then digested with collagenase II for 15 minutes at 37°C. After passing cells through a 100-µm cell strainer and centrifugation at 1,000 *g* for 10 minutes, primary adipocytes were collected from the top layer of the supernatant and the pellet containing the stromal vascular fraction (SVC) fraction was then incubated with red blood cell lysis buffer.

RNA isolation and q-PCR

Total RNA was isolated using TRIzol (Invitrogen, CA) according to the manufacturer's instructions. First-strand cDNA was synthesized using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). For q-PCR, samples were run in a 20-µl reaction (iTaq SYBRgreen supermix; BioRad, CA) using a stepOnePlus Real-Time PCR system (Applied Biosystems). Gene expression levels were calculated after normalization to the standard housekeeping gene *Actb* and RNA polymerase II, using the Ct method as described previously (48), and expressed as relative mRNA levels compared with internal control. Primer sequences available upon request.

Statistics

When comparing two groups statistical analysis was performed by a 1 or 2-tailed Student's *t* test. A two way ANOVA with repeated measures followed by a Tukey post hoc test was used to compare multiple groups at different time points using GraphPad Prism 6, unless otherwise stated. For the lipid analysis, significance was determined by one-way ANOVA on log10 transformed data and Benjamini and Hochberg was used for false discovery rate adjustment. Significance was defined as *q* value equal or less than 0.05. All data are expressed as mean ± SEM. "Reversibility" was defined as a significant change between the lean and obese state (*q* < 0.05 LFD v HFD), which then changes back after weight loss (*q* < 0.05 HFD v SWD), while the level after weight loss is comparable with the original lean state (*q* > 0.05 SWD v LFD). The "persistent" class contains metabolites that change significantly between the lean and obese state (*q* < 0.05 LFD v HFD), that do not significantly change after weight loss (*q* > 0.05 HFD v SWD) and the new level after weight loss is also significantly different from the original LFD level (*q* < 0.05 SWD v LFD). The third class, includes metabolites that are "not fully reversed" after weight loss, where the metabolite is significantly changed in the obese state (LFD v HFD), but after weight loss, the levels are not significantly different from the HFD level (HFD v SWD not significant), and the new level after weight loss is not significantly different from the lean state (SWD v LFD not significant). No blinding was carried out for data analysis.

Results

To identify lipid mediators that are regulated by obesity or weight loss we used a model of HFD feeding to induce obesity in C57BL6 mice and then switched the diet to LFD to induce weight loss (Fig 1A). Mice chronically fed HFD developed obesity (Fig 1B) and glucose intolerance (Fig 1C). Switching from HFD to LFD feeding for a further 9 weeks resulted in weight loss (Fig 1D) and improvement in glucose tolerance (Fig 1E) in the SWD group to a similar level as mice that were continuously fed LFD throughout the study.

Targeted lipid mediator analysis of endocannabinoids, oxylipins and ceramides in metabolic tissues including liver, adipose tissue, skeletal muscle, hypothalamus and plasma, identified 57 metabolites that were significantly induced or repressed in the obese state compared with lean controls (Fig 2). Of these changes the majority were increased in the HFD fed, obese state while only 8/57 lipids were lower in the HFD fed mice compared with LFD fed mice (Fig 2A). The change in expression levels of metabolites between the lean and obese state were classified as either “reversible”, “persistent”, or “not fully reversed” after weight loss. The majority of these changes (28/57, = 49%) were reversible after weight loss, while 12/57 (= 21%) are not fully reversed and (17/57, =30%) are persistent and do not revert back to lean state after weight loss (Fig.2B).

The majority of changes in lipid mediators occurred in the adipose (25/57 =43%) and liver tissues (21/57 = 36.8%), while far fewer were observed in the plasma (8/57=14%), muscle (2/57=3.5%) and hypothalamus (1/57=1.8%), (Fig 2C). After weight loss, induced by switching the diet from HFD to LFD, many of the obesity/HFD-induced changes observed in the adipose tissue were persistent and did not return to the equivalent LFD fed levels, while no persistent changes were observed in the liver, muscle, hypothalamus or plasma.

HFD feeding resulted in changes in concentration of lipids across multiple tissues (Fig 2D–F). For example, oxylipins such as 13-HODE, 9-HOTE, 9-HEPE and 9-HODE all increased in both adipose and liver from obese, HFD fed mice compared with lean LFD-fed mice (Fig 2D). Endocannabinoid-like compounds 1-OG, 2-LG and LEA were all increased in HFD fed mice compared with lean, LFD fed mice (Fig 2E). HFD feeding resulted in increased 1-OG in the adipose and liver; increased 2-LG in adipose and plasma; and increased LEA across adipose tissue, liver, hypothalamus and plasma compared with LFD fed mice. HFD feeding resulted in decreased OEA in the liver but increased OEA in the plasma compared with LFD-fed mice (Fig 2E). HFD resulted in increased C18:1 and C20 ceramide in adipose, muscle and plasma; increased C18 dihydroceramide in adipose and plasma and increased 18:1 sphingosine in adipose and liver compared with LFD fed mice (Fig 2F). Some of these changes were reversible upon weight loss, while some persisted at the level observed in the obese state (Fig 2D–F).

The individual lipids that are significantly different in HFD fed mice compared with LFD fed mice are classified into reversible, not fully reversible and persistent classes (Fig 3). The majority of changes induced by HFD feeding/obesity in the liver (Fig 3A) plasma, muscle and hypothalamus (Fig 3B) are reversible after weight loss. In contrast, the majority of the changes induced by HFD feeding in adipose tissue are persistent after weight loss (Fig 3C).

Of the 25 lipids that significantly change in adipose tissue after HFD feeding only 5/25 had fully reversed back to lean levels after weight loss, 3 were partially reversed while 17/25 were persistently high after weight loss. Of these persistent changes, prostaglandin signaling is perturbed with lasting increases in PGD2 and downstream metabolites Delta-12-PGJ2 and 15-deoxy PGJ2 (Fig. 3D & E) in adipose tissue. Notably other cyclooxygenase-dependent metabolites including TXB2, 6-keto PGF1 α and PGE2 (Fig 3E) were not induced by HFD feeding.

Cyclooxygenase-dependent metabolites, including the prostaglandins (PGs) and thromboxanes (TXs) have broad roles in both healthy and pathological contexts (51). Cyclooxygenase enzymes catalyze the conversion of arachidonic acid to PGH2, which is converted to species including PGD2, PGE2, PGF2 α , prostacyclin (PGI2) or TXA2 by the action of specific synthases (52). PGD2 is further dehydrated to produce PGJ2, delta 12-PGJ2, and 15-deoxy-delta 12,14-PGJ2. The synthesis of PGD2 from PGH2 can be catalyzed by two synthase enzymes termed prostaglandin D synthase (PTGDS) (53) and hematopoietic prostaglandin D synthase (HPGDS) (54). HPGDS is widely distributed in the peripheral tissues while PTGDS is highly expressed in the central nervous system, but also expressed at lower levels in other peripheral tissues (55). We therefore measured the expression of HPGDS and PTGDS enzymes in adipose tissue from these three diet groups. HPGDS was increased in HFD fed mice by ~10 fold compared with LFD fed mice and the expression level remained high in the SWD group (Fig. 4A), while PTGDS was unchanged between groups (Fig. 4B). To determine the cellular source of HPGDS in adipose tissue we fractionated the adipocytes and SVCs from LFD, HFD and SWD fed mice (Fig 4C). Levels of HPGDS were similar between adipocytes and SVCs from LFD fed mice. However, after HFD feeding the levels of HPGDS increased significantly in both adipocytes and SVCs compared with LFD fed mice and this increase persisted in both adipocyte and SVC fractions after weight loss (Fig 4C). In agreement with other studies (56), we also observed persistent expression of macrophage markers after weight loss (Fig 4 D and E).

To determine whether these findings were also conserved in human subjects we measured the levels of these enzymes in human adipose tissue biopsies taken from lean and obese patients. We previously performed microarray analysis on human adipose tissue biopsies (57) and mined this dataset to determine if HPGDS was differentially expressed between lean and obese individuals. HPGDS levels were increased ~1.95 fold compared with lean individuals (BMI 23.2 v 35.9) (Fig. 4F). This increase in HPGDS in adipose tissue from obese patients was also observed when we extracted the expression data from similar published studies (Fig 4G), NCBI GEO dataset GDS3679 (58). We then extracted data from studies comparing expression levels in adipose tissue before and after weight loss (59, 60) and looked at HPGDS levels to see if obesity-induced increase in HPGDS was maintained after weight loss as seen in our mouse studies. In support of the mouse data, diet induced weight loss of up to 15% (Fig 4H) (59), or after a very low calorie diet for 16wks with average weight loss of 27.7 kg (Fig 4I) (60) did not change the expression levels of HPGDS in human adipose tissue. We also measured circulating levels of PGD2 in obese subjects before and after gastric bypass surgery (Fig 4J–K). Circulating levels of PGD2 in obese patients were approximately 2 fold higher than the clinical reference range for normal individuals (57, 61–64). Furthermore, after substantial weight loss of ~35% (average BMI

reduced from 43.6 to 28.2) PGD2 levels were unchanged one year after gastric bypass surgery.

Due to the persistent elevation of PGD2 and HPGDS we hypothesized that PGD2 may play a role in food intake. Central injection of PGD2 has previously been shown to stimulate food intake (65) but whether peripheral levels of PGD2 could play a role in food intake is not yet known. To reduce peripheral levels of PGD2 we treated mice with the selective HPGDS inhibitor HQL-79 (66). Previous studies have shown that HQL-79 selectively inhibits HPGDS while not affecting PTGDS and results in lowering of PGD2 levels (67–69). Treatment of wild type, HFD fed mice with HQL-79 (30mg/kg, oral gavage) for 5 days resulted in a significant reduction in food intake (Fig 5A), with an associated trend in reduction of body weight ($p=0.09$) (Fig 5A). Whole adipose tissue levels of PGD2 in whole adipose tissue were not significantly different between the control and HQL-79 treatment groups, but the ratios between PGD2 and other connected pathway metabolites indicate a shift toward the production of PGE2 and PGF2a, in addition to their metabolites, instead of PGD2 (Supplemental figure 1) after HQL-79 treatment.

Discussion

We hypothesized that lasting changes in the metabolome after weight loss may play a role in feeding behavior and contribute to weight regain. We characterized changes in the levels of oxylipins, endocannabinoid and ceramide lipid mediators in lean, obese and weight-reduced mice. We identified lasting changes in the lipid mediators in the weight-reduced mice and identified many persistent changes specifically in adipose tissue. Of these persistent changes, prostaglandin signaling was perturbed with persistent elevation in PGD2 and downstream metabolites 15-deoxy PGJ2 and delta-12-PGJ2. In addition, levels of the enzyme responsible for PGD2 synthesis (HPGDS) were also increased in adipose tissue in the obese state and did not decrease after weight loss in both mouse and human studies.

PGD2 plays a broad role in many biological processes including sleep (70), analgesia (71) allergic diseases (52, 72) and food intake, where injection of PGD2 directly into the brain increases food intake in mice (65). Prostanoids exert a wide variety of actions in the body, which are mediated by specific receptors on plasma membranes (73). PGD2 acts through two receptors (DP1 and DP2 CRTH2), whereas 15-deoxy PGJ2 can activate some peroxisome proliferator-activated receptors and inhibit proinflammatory pathways (74). Our finding that levels of prostaglandin signaling increased in obesity are in agreement with previous published studies showing elevated PGD2 and HPGDS in mouse adipose tissue (75). Furthermore, transgenic overexpression of H-PGDS in mice results in obesity and increased fat mass (76). We confirm and extend these findings by showing this obesity-induced increase in PGD2 does not decrease after weight loss and may play a role in body weight regulation.

In human studies, prostaglandin signaling has been linked to obesity in correlation studies showing that expression of the prostaglandin synthesis enzyme PTGDS in the cerebrospinal fluid significantly correlated with levels of orexigenic neuropeptides (77). In addition, PTGDS levels in CSF are positively correlated with visceral adipose tissue and negatively

correlated with subcutaneous adipose tissue mass (77). In our studies we have mined expression databases of human adipose tissue from lean and obese subjects and found levels of HPGDS were elevated in the obese state and did not decrease after weight loss. In addition circulating levels of PGD2 were elevated compared with the lean reference range, and gastric bypass-induced weight loss did not decrease circulating PGD2 levels.

The lasting changes in prostaglandin signaling in adipose tissue after weight loss suggest a possible role in weight regain. Previous studies have shown that central injection of PGD2 stimulates food intake, (65) but whether peripheral levels of PGD2 could play a role in food intake was not yet known. We show that inhibition of HPGDS (by oral gavage of HQL-79) results in decreased food intake and thus could be a therapeutic strategy in prevention of weight regain after weight loss. After 5 days of HQL-79 administration in HFD fed mice the adipose tissue ratios between PGD2 and other connected pathway metabolites indicate a shift toward PGE2 and PGF2a production instead of PGD2 after HQL-79 treatment (Supplemental fig 1A), however, detected levels of PGD2 were not significantly different in whole adipose tissue. Fractionating the adipocyte and SVF would determine whether either cell type up-regulate PGD2 production in response to HPGDS inhibition (67) but the isolation procedure itself will likely cause alterations in metabolite levels that would no longer reflect the in vivo state (75) due to rapid degradation of PGD2 within a few minutes (78). Furthermore, although we give the H-PGDS inhibitor peripherally, we cannot rule out the possibility that this inhibitor may also act directly on the brain to reduce PGD2 levels which may also play a role in the observed effects on food intake. Future studies are needed to fully understand the role of adipocyte and SVC PGD2 levels on the regulation of food intake and energy expenditure. There are currently specific HPGDS inhibitors being developed for other indications including asthma and chronic obstructive pulmonary disease (79, 80). Our data strongly suggests that inhibition of HPGDS may also be beneficial to maintain weight loss and prevent weight regain.

The role of adipose tissue in the control of food intake is well established, with leptin being the most well known example (81), but how peripheral levels of PGD2 may affect feeding behavior is still unknown. Body weight is controlled centrally by the hypothalamus which integrates signals emanating from the periphery including gastrointestinal, hepatic, and adipose tissue to control food intake and energy expenditure (82) (83). PGD2 from adipose tissue may travel via the systemic circulation, crossing the blood brain barrier and directly reach the brain (82). However, we do not observe a significant increase in PGD2 levels in the plasma of obese mice, which may be due to instability of this metabolite that has a half-life in the blood of less than 2 mins (78), (Supplemental Table 1). Alternatively, the signal from adipose to brain may be transduced by changes in the firing rate of sensory nerve fibers. Viral tracing studies have identified afferent circuits projecting from WAT to the central nervous system, supporting the existence of a sensory WAT pathway to the brain (84, 85). Therefore, further investigation is needed to understand the mechanism by which adipose tissue levels of lipid mediators may regulate food intake.

Despite the persistent metabolic adaptations that act to proportionally counter efforts to reduce body weight (86), some studies report that weight loss maintenance gets easier over time: after individuals have successfully maintained weight loss for 2–5yrs the chances of longer

term success greatly increases (9). Human adipocytes turn over very slowly, and on average are only replaced every 10 years (87, 88). Therefore, changes induced in adipocytes by obesity may be longer lasting than in cell types that are replaced more readily. In addition, the obesity-induced increase in macrophage infiltration into adipose tissue does not reverse after weight loss and these cells are still present in adipose tissue after sustained weight loss in mice (56). Therefore, the “metabolic memory” of adipose tissue is difficult to erase even after maintenance of weight loss. In future time course studies, it will be interesting to see if the persistent changes in the adipose metabolome will eventually reverse after a longer time frame.

These studies reveal that obesity induces a lasting metabolic signature that does not resolve after weight loss. This metabolic signature resides predominantly in adipose tissue while other metabolic tissues including liver, muscle and hypothalamus return to pre-obese levels after weight loss. We show that PGD2 signaling is implicated in food intake and that high levels of HPGDS and circulating PGD2 are still present in human subjects despite weight loss. The metabolic characteristics of adipose tissues after weight loss could play an important role in the persistence of a biological drive to regain weight.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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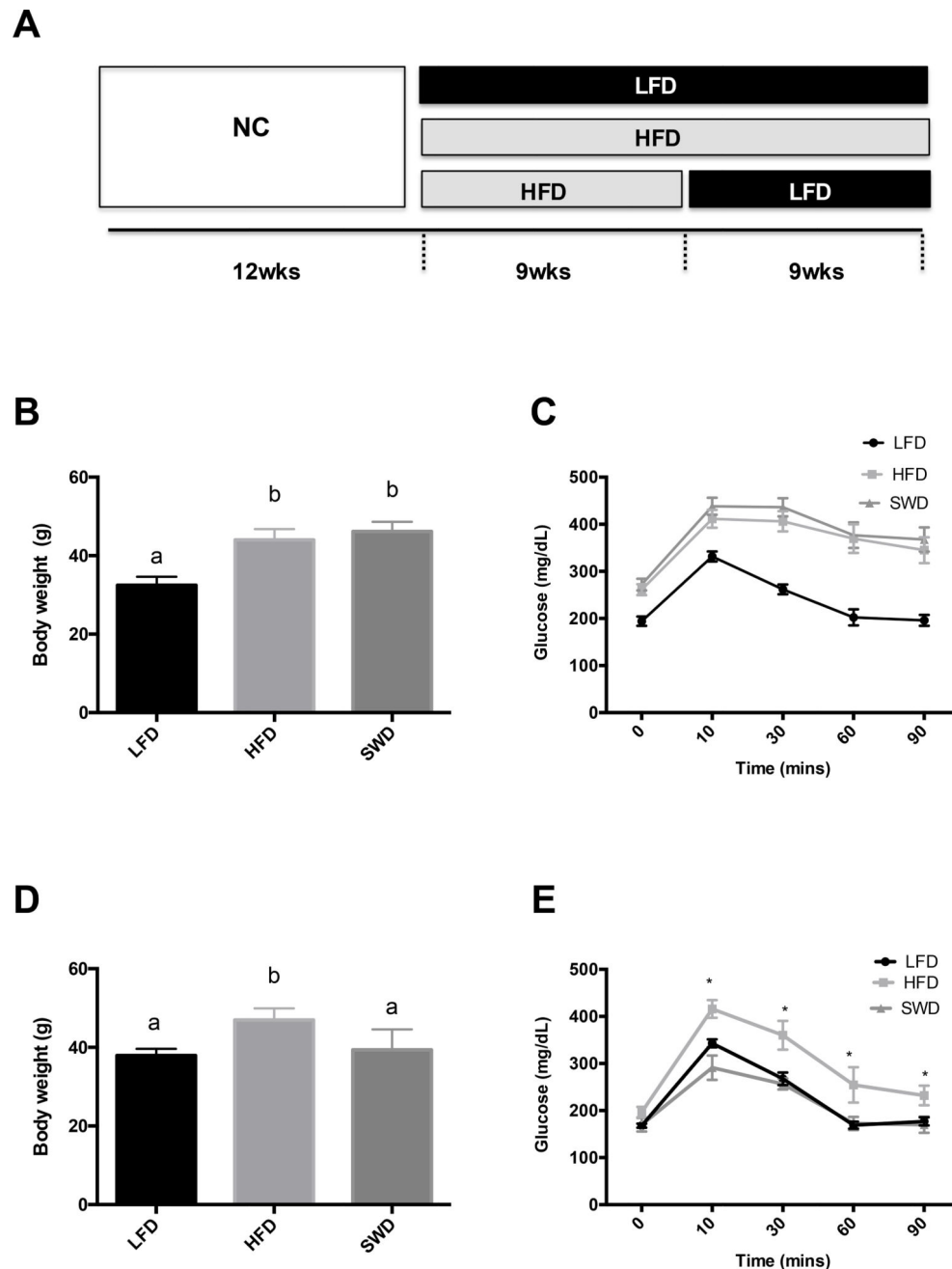


Figure 1. Model of obesity and weight loss in mice

A) Male C57BL6 mice were fed normal chow (NC) until 12 weeks of age, and then divided into three groups fed either low fat diet (LFD) or high fat diet (HFD) for a further 18 weeks, or HFD for 9 weeks and then switched to LFD for 9 weeks to induce weight loss (SWD). B) Body weights after 9 wks of LFD or HFD feeding. C) GTT after 9 weeks of feeding HFD or LFD. D) Body weights 8 wks after the diet switch E) GTT, 8 weeks after the diet switch. Significance was defined as p-value equal or less than 0.05. *, values labeled with different letters are significantly different.

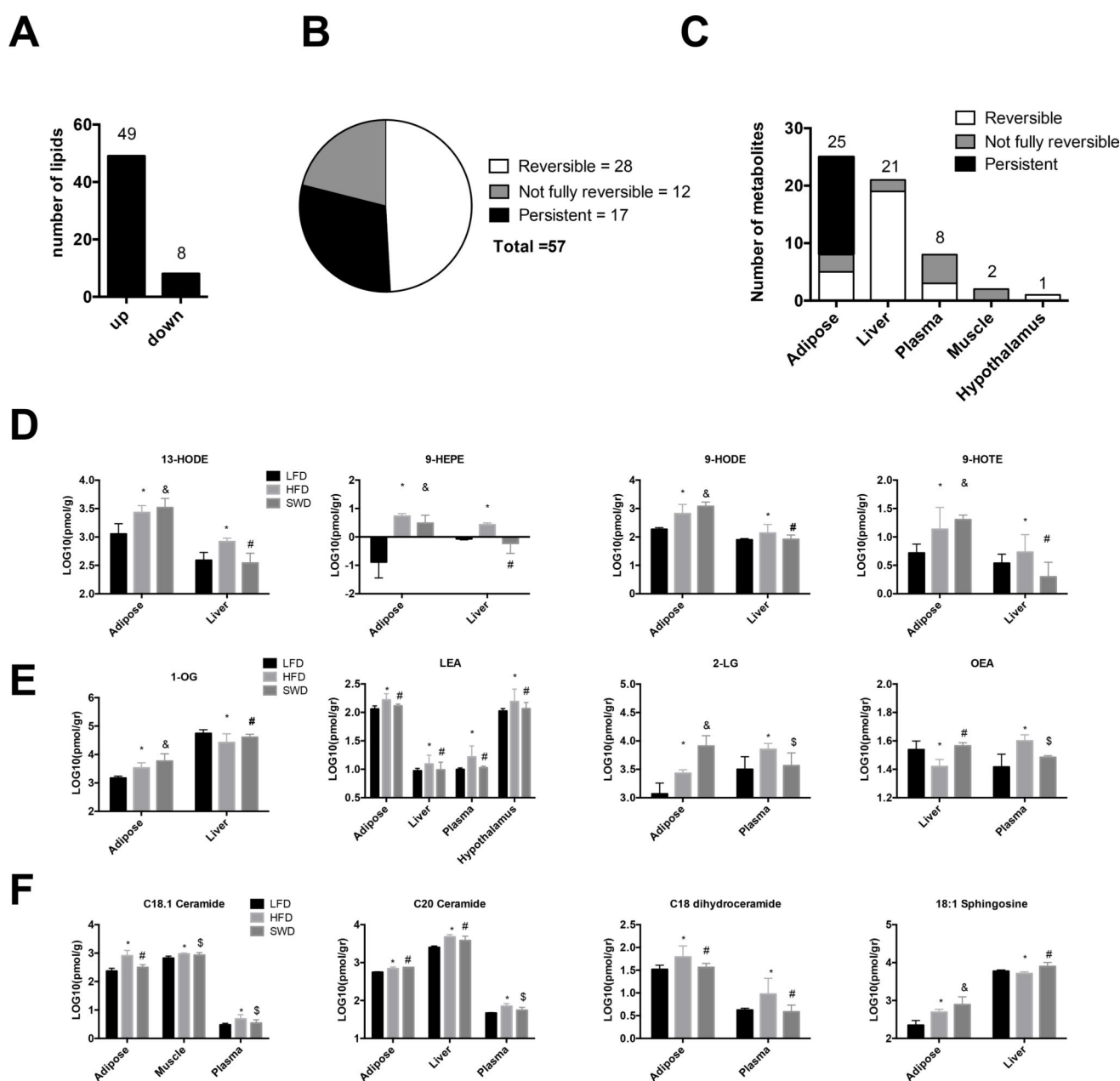


Figure 2. Targeted analysis of lipid mediators: Oxylipins, Endocannabinoids and Ceramides

A) Changes in lipid mediators observed between the lean (LFD) and obese (HFD) state across all tissues (liver, adipose, skeletal muscle, hypothalamus and plasma). B) The effect of weight loss on lipid mediators that were significantly changed in the obese state versus lean; defined as reversible, persistent and not fully reversible after weight loss induced by LFD feeding. C). Number of metabolites that are reversible, persistent, or not fully reversible within each tissue. Concentration of D) Oxylipins, E) Endocannabinoids and F) Ceramides. Lipid analysis significance was determined by One-Way ANOVA on log10 transformed data and Benjamini and Hochberg false discovery rate was used for multiple comparison adjustments. All data are expressed as mean \pm SEM. Significance was defined as q-value equal or less than 0.05. *, Significant change between LFD and HFD, # reversible

change after LFD feeding, \$, not fully reversible after LFD feeding, & persistent change after diet switch.

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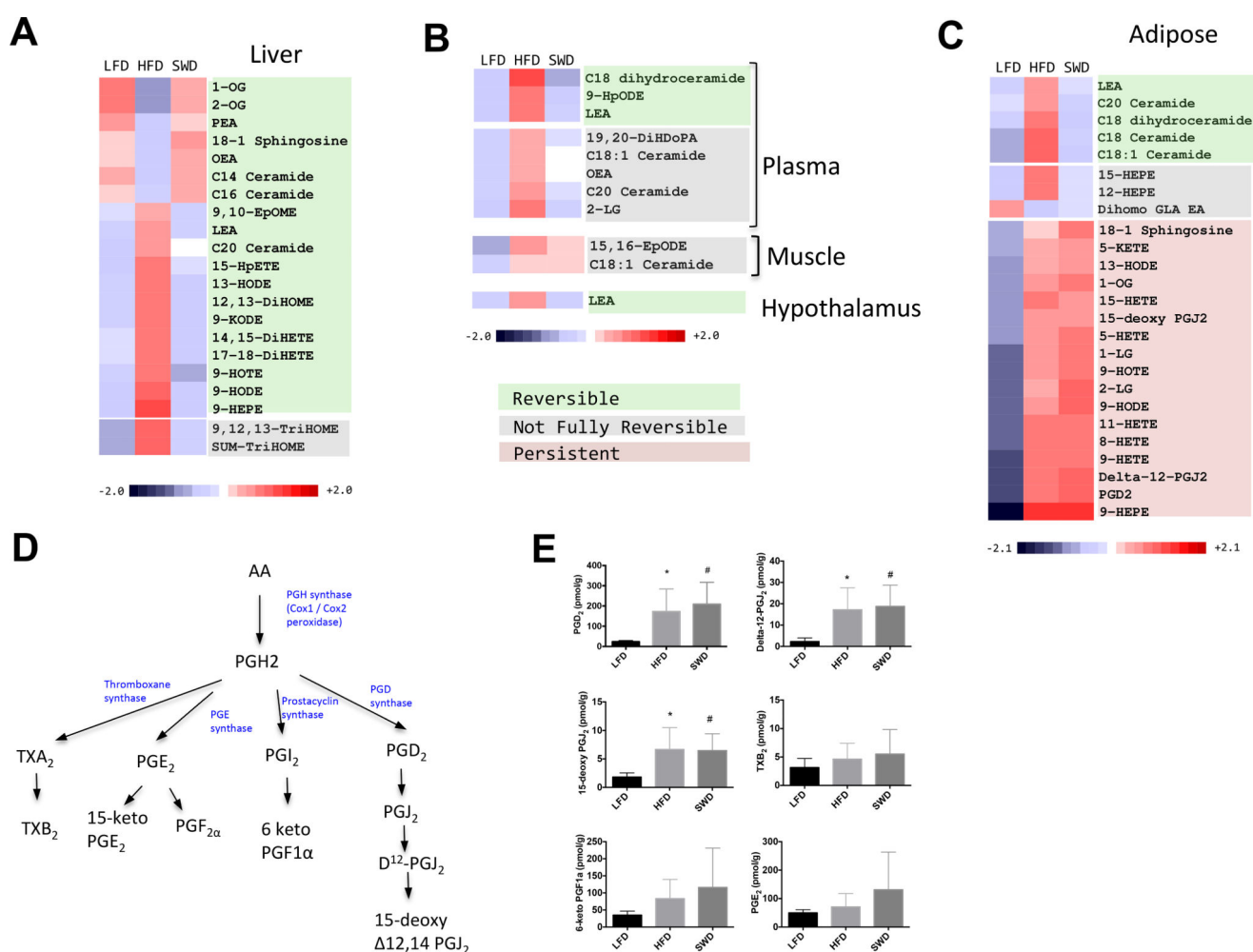


Figure 3. Persistent increase in PGD2 levels after weight loss

Heatmaps compare levels of lipid mediators across the three diet groups (LFD, HFD and SWD). A) Liver. B) Plasma, muscle and hypothalamus. C) Adipose. Lipids shaded green are reversible, grey = not fully reversible, orange = persistent. Fold change is reflected by intensity of color blue (decreased) to red (increased). D) Pathway of prostaglandin synthesis. Cyclooxygenase enzymes catalyze the conversion of arachidonic acid to PGH₂, which is converted to other species including PGD₂, PGE₂, PGF_{2α}, prostacyclin (PGI₂) and thromboxane (TX) A₂ by the action of specific synthases. E) Prostaglandin levels in lean (LFD), obese (HFD fed) and after weight loss (SWD).

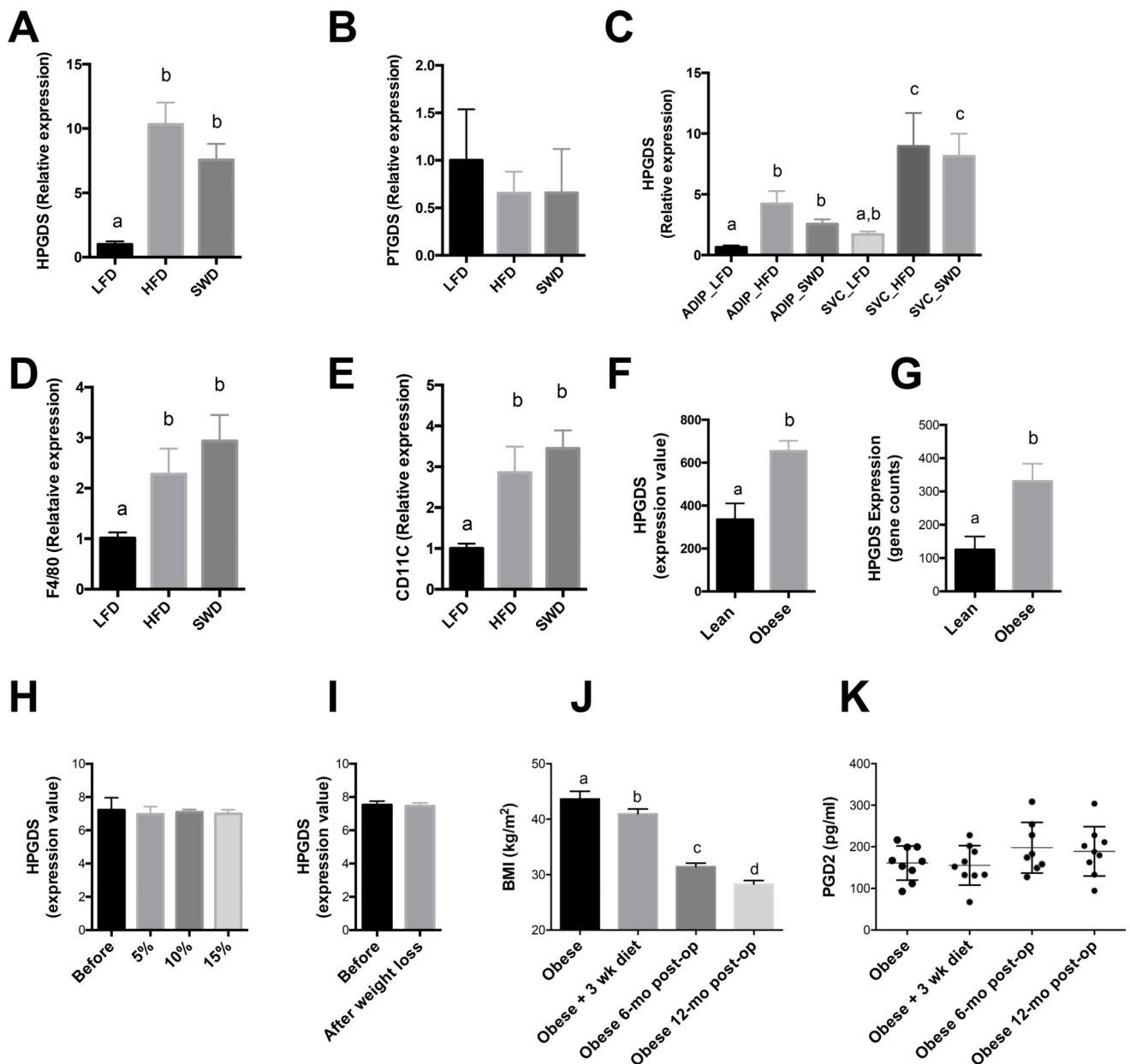


Figure 4. Obesity-induced increase in prostaglandin D2 signaling does not decrease after weight loss

A) Relative expression of HPGDS in adipose tissue from LFD, HFD and SWD mice. B) Relative expression of PTGDS in adipose tissue from LFD, HFD and SWD mice. C) HPGDS expression in adipocyte and stromal vascular cells (SVC) from LFD, HFD and SWD mice. D) F4/80 and E) CD11c expression in SVC from LFD, HFD and SWD mice. F) Expression of H-PGDS in adipose tissue biopsies from lean (n=6, BMI 23.2 ± 0.8) and obese (n= 35, BMI 35.9 ± 1.1) individuals, * q < 0.05 (57). G) Lean and obese omental adipose tissue, NCBI GEO dataset GDS3679 (58), 1 –tailed t-test, q < 0.1. H) Before and after weight loss of 5, 10 and 15% (59). I) Before and after weight loss induced by very low calorie diet for 16wks (60). J) BMI of patients before and after bariatric surgery. K) Plasma PGD2 levels in obese patients before and after gastric bypass surgery. Significance was

defined as q-value equal or less than 0.05. *, values labeled with different letters are significantly different.

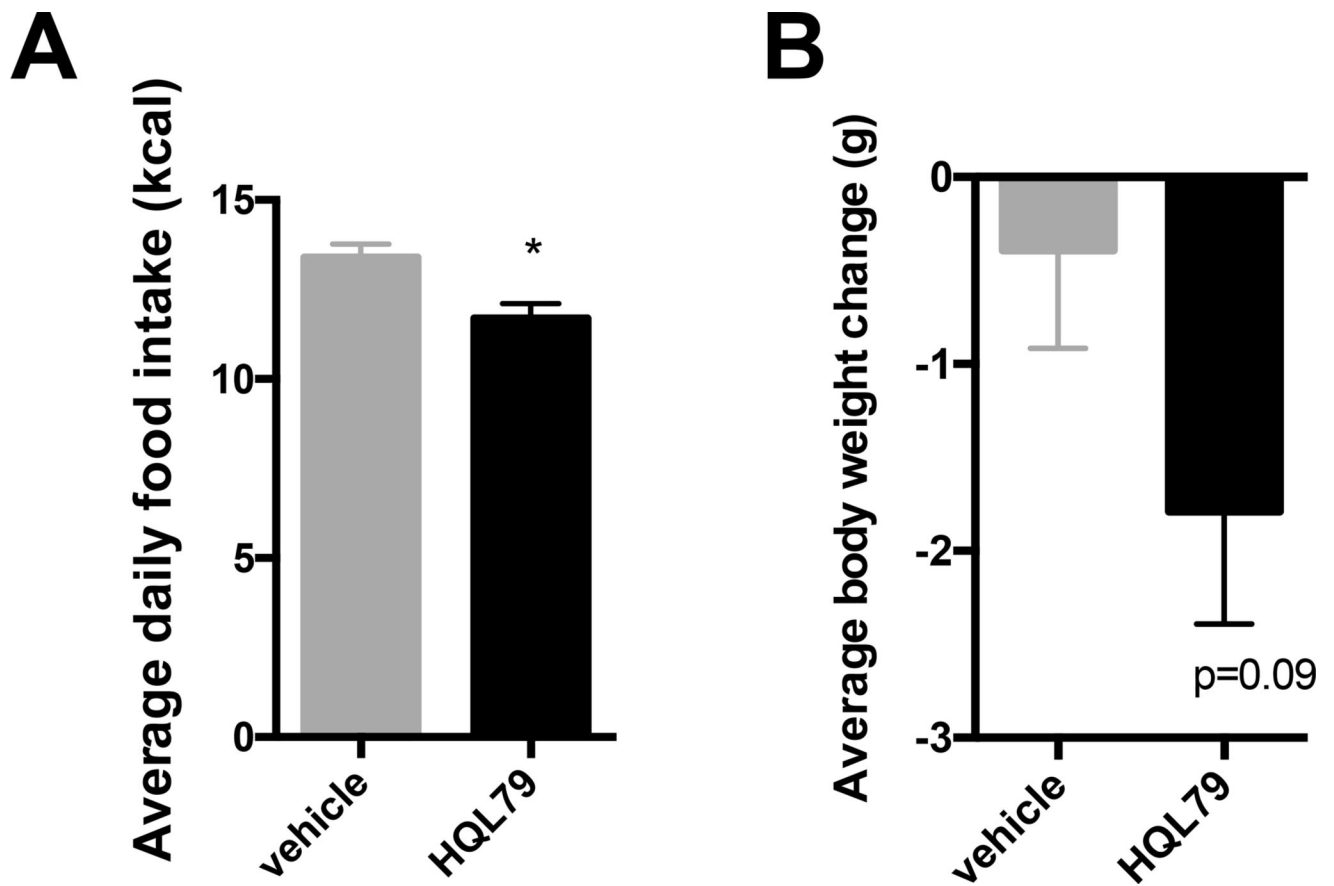


Figure 5. Food intake and body weight changes in mice treated with HPGDS inhibitor
A) Average daily food intake (kcal) and B) body weight change in HFD fed C57BL6 male mice treated with HPGD2 inhibitor (HQL-79, 30 mg/kg, daily oral gavage) for 5 days (n=12 per group).